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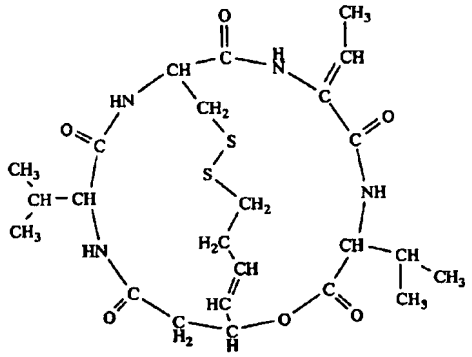
(54) POTENTIALISATEUR D'EXPRESSION GENETIQUE
(54) GENE EXPRESSION POTENTIATOR

(57)

An expression potentiator or reactivation promoter of a transgene, which contains FR901228 of the formula (I) <IMG> or a salt thereof as an active ingredient. FR901228 or a salt thereof used in the present invention as an active ingredient shows not only in vitro but in vivo superior potentiation of a expression of a transgene and promotion of the reactivation of the transgene. Therefore, it can be advantageously used in clinical situations, particularly for a gene therapy.



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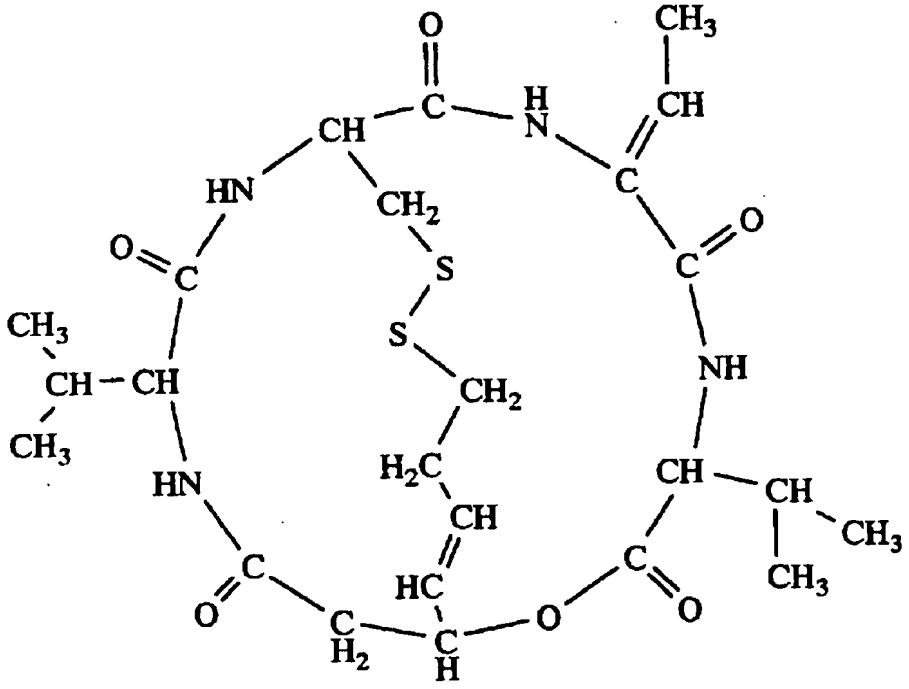

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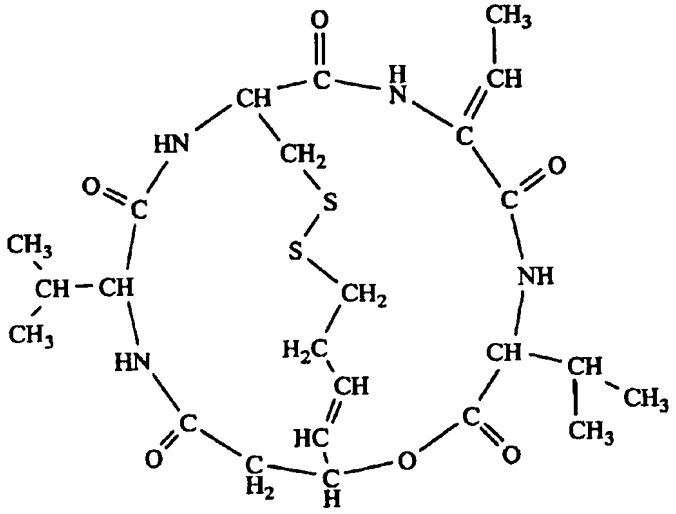
(57) Abrégé/Abstract:
An expression potentiator or reactivation promoter of a transgene, which contains FR901228 of the formula (I) (see formula I) or a salt thereof as an active ingredient. FR901228 or a salt thereof used in the present invention as an active ingredient shows not only in vitro but in vivo superior potentiation of a expression of a transgene and promotion of the reactivation of the transgene. Therefore, it can be advantageously used in clinical situations, particularly for a gene therapy.

ABSTRACT OF THE DISCLOSURE

An expression potentiator or reactivation promoter of a transgene, which contains FR901228 of the formula (I)



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or a salt thereof as an active ingredient. FR901228 or a salt thereof used in the present invention as an active ingredient shows not only *in vitro but in vivo* superior potentiation of a expression of a transgene and promotion of the reactivation of the transgene. Therefore, it can be advantageously used in clinical situations, particularly for a gene therapy.

SPECIFICATION

GENE EXPRESSION POTENTIATOR

TECHNICAL FIELD OF THE INVENTION

The present invention relates to an expression potentiator and reactivation promoter of a transgene. More particularly, the present invention relates to an expression potentiator and reactivation promoter of a transgene, which contains a compound having a histone deacetylase inhibitory activity.

BACKGROUND OF THE INVENTION

Gene therapy is considered to be effective for the treatment of intractable diseases, and more than 300 protocols of the gene therapy have been applied in the US (as of June 1999). However, the therapy has proved effective only in a few cases, thereby highlighting the following essential problems that the gene therapy should overcome before providing successful results.

(1) Development of a vector much superior in the efficiency of gene transfer.

(2) Enhanced efficiency of gene transfer into non-proliferative cells.

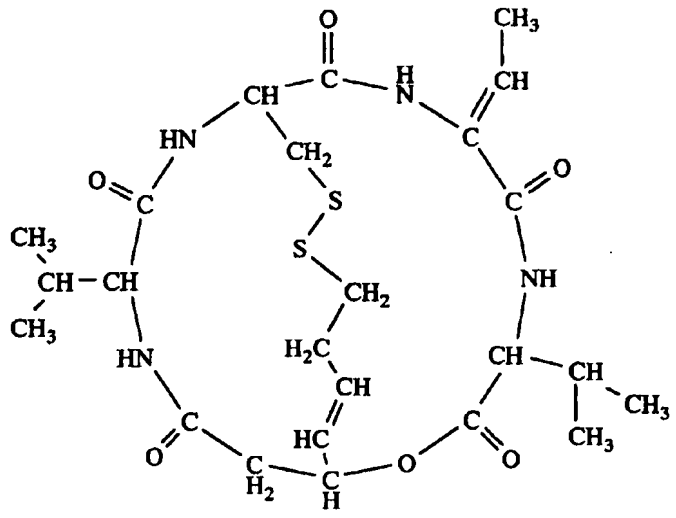
(3) Realization of sustained high expression of a transgene.

The first and the second problems are being dealt with by the development of a liposome vector, adenovirus vector and the like. On the other hand, the third problem, though very essential for a method of therapy, is far behind in its development. The problem stems from the low expression or suppressed expression of the transgene in clinical situations. For a transgene to be expressed in human cells in a sustained manner, a stable integration of the gene into the genome of the host cell is essential. However, exogenous genes after incorporation into the genome are reportedly highly susceptible to the suppression of expression (silencing). The silencing of the gene incorporated into the genome is postulated to be caused by the changes in the genomic structure (structural change of chromatin) at the incorporation site, which then suggests a possibility of involvement of acetylation and deacetylation of histone. This silencing has been elucidated to be also found in the plasmid DNA

that has not been incorporated into the genome but present in the nucleus. In recent years, the use of a specific inhibitor of histone deacetylase has become available. The histone deacetylase inhibitor leads chromatin to a hyper acetylation state by the suppression of deacetylation of histone. Using a histone deacetylase inhibitor, therefore, the relationship between the hypoacetylation state of histone and transcription inhibition state, and hyperacetylation of histone and activation of transcription has been elucidated, whereby the importance of the acetylation and deacetylation of histone in the regulation of transcription is being empirically established.

Recent reports have documented the reactivation of transcription (release of silencing) of a transgene *in vitro* using a histone deacetylase inhibitor (Dion, L. D. et al., Virology 231, 201-209 (1997); Chen, W. Y. et al., Proc. Natl. Acad. Sci. USA 94, 5798-5803 (1997)). This suggests the resolution of the third problem by a drug. As the histone deacetylase inhibitor, trichostatin A, sodium butyrate and the like have been reported, but due to the poor absorption and behavior in blood, or due to the insufficient action, there is no report on the effect *in vivo*, and a clinical utilization, particularly application to a gene therapy, has been considered to be unfeasible. In addition, there is no report on the activation of transcription by a histone deacetylase inhibitor at an individual level, which denies clinical utility.

Incidentally, a compound of the following formula (I)



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has been reported to have a histone deacetylase inhibitory activity (Nakajima, H. et al., Experimental Cell Research 241, 126-133 (1998)), and therefore, is useful clinically as an anticancer agent (US Patent No. 4,977,138). However, this compound is unknown as to the influence on the expression and reactivation of a transgene.

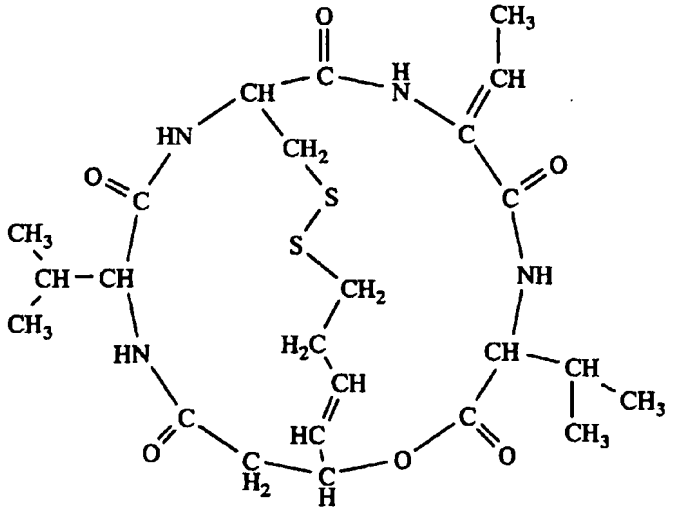
SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide an expression potentiator and reactivation promoter of a transgene that enables sustained expression of a transgene.

Another object of the present invention is to provide an expression potentiator or reactivation promoter of a transgene, which can be used as a drug, particularly preferably for a gene therapy.

Such object can be achieved by the present invention described in the following.

According to the present invention, there is provided a compound of the following formula (I)



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(hereinafter to be also referred to simply as FR901228), or a salt thereof that shows not only *in vitro* but also *in vivo* superior potentiation of expression of a transgene and promoted reactivation of a transgene. That is, the present invention is as follows.

(1) An expression potentiator or reactivation promoter of a transgene, which contains a compound of the formula (I) or a salt thereof as an active ingredient.

(2) The potentiator or promoter of (1) above, which potentiates expression or promotes reactivation of a transgene *in vivo*.

(3) The potentiator or promoter of (1) or (2) above, which is a drug.

(4) The potentiator or promoter of (3) above, wherein the drug is for a gene therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A shows dose-dependent potentiation by FR901228 of the expression of luciferase gene in B16-F1 cells, wherein the ordinate shows the ratio of luciferase activity with the administration of FR901228 to luciferase activity without the administration of FR901228 and the abscissa shows the concentration of FR901228.

Fig. 1B shows dose-dependent potentiation by FR901228 of the expression of luciferase gene in NIH3T3 cells, wherein the ordinate shows the ratio of luciferase activity with the administration of FR901228 to luciferase activity without the

administration of FR901228 and the abscissa shows the concentration of FR901228.

Fig. 2 shows potentiation by FR901228 of the expression of the luciferase gene in B16-F1 cells with the lapse of time, wherein the ordinate shows luciferase activity and the abscissa shows the lapse of time after transfection.

Fig. 3 shows dose-dependent reactivation by FR901228 of the expression of the luciferase gene in B16/L cell where the luciferase gene has been incorporated into the genome, wherein the ordinate shows the ratio of luciferase activity with the administration of FR901228 to luciferase activity without the administration of FR901228 and the abscissa shows the concentration of FR901228.

Fig. 4 shows potentiation of the expression of the transduced gene in mice by the transfection of the luciferase gene in the presence of FR901228.

Fig. 5 shows reactivation of the expression of the luciferase gene in mice by FR901228.

Fig. 6 shows potentiation of the expression of the transduced gene in mice by the transfection of the luciferase gene in the presence of various doses of FR901228.

Fig. 7 shows potentiation of the tumor growth inhibitory activity of gancyclovir in mice by the transfection of the thymidine kinase gene in the presence of FR901228, wherein the ordinate shows tumor volume and the abscissa shows the number of days elapsed after transplantation of B16-F1 cells.

DETAILED DESCRIPTION OF THE INVENTION

The inventive expression potentiator and reactivation promoter of a transgene contains a compound of the formula (I) or a salt thereof as an active ingredient. This compound has a potent histone deacetylase inhibitory activity (Nakajima, H. et al., *supra* (1998)).

The compound of the formula (I) can be produced by culturing a bacterial cell belonging to the genus *Chromobacterium* such as *Chromobacterium violaceum* WB968 strain (FERM BP-1968) and the like, that produces a compound of the formula (I) (FR901228), in a nutrient medium. More specifically, it can be produced from the above-mentioned production strain according to the method

described in US Patent No. 4,977,138. FR901228 can be produced by a fermentation method which comprises harvesting from culture of the above-mentioned bacterial cell belonging to the genus *Chromobacterium*. Alternatively, it can be semi-synthesized or completely synthesized according to a method conventionally known. More specifically, the method reported by Khan W. Li, et al. (J. Am. Chem. Soc., vol. 118, 7237-7238 (1996)) can be used.

The salt of FR901228 is a biologically acceptable salt that is generally non-toxic, and is exemplified by salts with base and acid addition salts, inclusive of salts with inorganic base such as alkali metal salt (e.g., sodium salt, potassium salt and the like), alkaline earth metal salt (e.g., calcium salt, magnesium salt and the like), ammonium salt, salts with organic base such as organic amine salt (e.g., triethylamine salt, diisopropylethylamine salt, pyridine salt, picoline salt, ethanolamine salt, triethanolamine salt, dicyclohexylamine salt, N'N'-dibenzylethylenediamine salt and the like), inorganic acid addition salt (e.g., hydrochloride, hydrobromide, sulfate, phosphate and the like), organic carboxylic sulfonic acid addition salt (e.g., formate, acetate, trifluoroacetate, maleate, tartrate, fumarate, methanesulfonate, benzenesulfonate, toluenesulfonate and the like), salt with basic or acidic amino acid (e.g., arginine, aspartic acid, glutamic acid and the like), and the like.

FR901228 sometimes has an optical isomer due to an asymmetric carbon and a double bond, and a stereoisomer of geometric isomer and the like, all of which and mixtures thereof are also encompassed in the present invention.

Further, solvate compounds (e.g., inclusion compound such as hydrate and the like) of FR901228 and a salt thereof are also encompassed in the present invention.

In the present invention, *in vivo* and *in vitro* means as these terms are used in this field. That is, "*in vivo*" means that the target biological functions and responses are expressed within tissues of the living body, and "*in vitro*" means that such functions and responses are expressed in the tissue culture system, cell culture system, non-cell system and the like.

In the present invention, potentiation of the expression of a transgene means potentiation in the host cell of the expression

of an exogenous gene transduced by genetic engineering into the cells of human and various animals (e.g., mouse, rat, swine, dog, horse, cow and the like). The potentiation of the expression of the transgene may be at the cell level (i.e., *in vitro*) or at an individual level (i.e., *in vivo*), with preference given to that *in vivo*.

In the present invention, reactivation of a transgene means release of the suppression of the expression (silencing) of an exogenous gene transduced by genetic engineering into the cells of human and various animals (e.g., mouse, rat, swine, dog, horse, cow and the like), and the present invention can promote the reactivation. Besides the release of silencing, the present invention can promote transcription activity of a transgene that shows stable expression at a constant level, and potentiate the expression. Such effect is also encompassed in the "reactivation of the transgene" of the present invention. The promotion of the reactivation of the transgene may be at a cell level (i.e., *in vitro*) or at an individual level (i.e., *in vivo*), with preference given to that *in vivo*.

An exogenous gene can be transduced by a method known in the pertinent field. For example, transfer of DNA by physical method (microinjection method, electroporation method and the like), transfer of DNA by chemical method (calcium phosphate method, DEAE-dextran method, liposome method etc.), biological method (virus vector such as retrovirus and adenovirus, and the like), new methods such as HVJ-liposome method and the like can be beneficially used.

The inventive expression potentiator and reactivation promoter of a transgene can be used as a solid, semi-solid or liquid pharmaceutical preparation containing FR901228 or a salt thereof as an active ingredient in admixture with an organic or inorganic carrier or excipient suitable for oral or parenteral application. The active ingredient can be admixed with a typical, non-toxic pharmaceutically acceptable carrier suitable for the dosage form, such as powder, tablet, pellet, capsule, suppository, liquid, emulsion, suspension, aerosol, spray and other form for use. Where necessary, auxiliary agent, stabilizer, tackifier and the like may be used. These carriers and excipients may be

sterilized where necessary, or a sterilization treatment may be applied after formulation into a preparation. FR901228 and a salt thereof are contained in the expression potentiator or reactivation promoter in an amount sufficient to produce a desired effect on the condition that requires potentiation of the expression of a transgene or reactivation thereof. In particular, when the inventive expression potentiator and reactivation promoter of a transgene is used for a gene therapy, parenteral administration is preferable, namely, intravenous administration, intramuscular administration, direct administration into the tissue, intra-nostril cavity administration, intradermal administration, administration into cerebrospinal fluid, administration into biliary tract, intravaginal administration and the like. In addition, a liposome method capable of direct administration to the site and organ where expression and reactivation of a transgene are requested, and the like can be preferably used.

The therapeutically effective amount of the active ingredient FR901228 and a salt thereof varies and is determined depending on the age and condition of individual patient to be treated, the kind of the transgene, and the kind of a disease where potentiation of the expression and promotion of reactivation of a transgene are requested.

For example, in the case of intravenous infusion administration, the daily dose for an adult is preferably 1 mg/m^2-50 mg/m^2, more preferably 3 mg/m^2-30 mg/m^2, which is administered once a day.

The administration method of the expression potentiator and reactivation promoter of a transgene of the present invention is free of any particular limitation as long as it can provide the effect of potentiation of the expression of a transgene and promotion of the reactivation of a transgene. When it is used in the form of a pharmaceutical preparation, it can be administered orally or parenterally once a day or several times a day. When it is used for a gene therapy, the administration route most suitable for the expression and reactivation of the transgene is appropriately selected in consideration of the specific nature of use. For example, when it is used for a gene therapy of tumor,

direct administration to the tumor cell (e.g., liposome method) is preferable.

The present invention is characterized by the potentiation of the expression of a transgene, as well as release of the suppression of the transgene expression, wherein the interaction with the transgene is an important factor for the exertion of the effect. Therefore, the timing of the administration of the transgene and the administration (*in vivo*, *in vitro*) to the subject of the expression potentiator or reactivation promoter of the present invention are appropriately determined according to the desired effect. When the potentiation of the expression of a transgene is aimed, for example, the inventive transgene expression potentiator is preferably administered along with or after the administration of the transgene. When the promotion of the reactivation of a gene already transduced is aimed, the inventive transgene reactivation promoter is preferably administered when the reactivation is needed after the administration of the transgene. When the expression potentiator or reactivation promoter of a transgene of the present invention is to be administered after the administration of the transgene, the timing of the administration is appropriately determined according to the desired effect and its level, state of expression of the gene previously transduced (level of expression, position of the transgene and the like).

In particular, the present invention can be beneficially applied to a gene therapy. For the gene therapy of cancer, for example, transfer of a suicide gene, DNA vaccine and the like can be applied. As the transfer of a suicide gene, there is exemplified transfer of cytosine deaminase (enzyme to convert an anticancer agent, 5-fluorocytosine (5-FC) from an inactive type to an active type compound) gene into cancer cells. The expression of this gene in a cancer cell can be potentiated by the present invention (induction of anti-tumor effect by cancer cell-specific and efficient conversion of 5-FC to an active type 5-FC). Alternatively, thymidine kinase (HSV-TK) gene of herpes simplex virus is transduced to a cancer cell, and then gancyclovir (GCV), which is an antiviral drug, is administered. The phosphorylated GCV generated by HSV-TK inhibits DNA synthesis of the cancer cell

and exerts the anti-tumor effect.

As the DNA vaccine, there is exemplified a tumor-associated antigen gene specifically expressed in a cancer cell. Transfer of the gene to a cancer patient, or reactivation of an endogenous tumor-associated antigen gene, expression of which is suppressed, or both of them, provide potentiation of the expression of the function of the tumor-associated antigen gene, which in turn enhances the immunity to the cancer of the patient.

In a gene therapy of cancer, there are also used p53 gene, cytokine gene (e.g., IL2, IL12 gene), antisense gene (K-ras antisense) and the like. For the gene therapy of cystic fibrosis, CFTR gene can be used and for the gene therapy of hemophilia, a coagulant factor gene can be used.

The present invention is explained in more detail in the following by way of Examples. It is needless to say that the present invention is not limited by these examples.

Example 1

1. Test material and test method

(1) Drugs

FR901228 isolated and purified according to the disclosure in US Patent No. 4,977,138 was used. For *in vivo* tests, FR901228 was dissolved in and diluted with balanced salt solution (BSS, 137 mM NaCl, 5.4 mM KCl, 10 mM Tris-HCl, pH 7.6) before use. This solution was also used for the preparation of a liposome (BSS/FR901228 solution). For the *in vitro* test using cells, FR901228 was dissolved in and diluted with a culture medium (Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin (50 units/ml) and streptomycin (50 μg/ml)) containing 10% fetal bovine serum before use.

(2) Vector

A pCMV-luci vector was constructed by binding a BamHI-XhoI restriction fragment of firefly luciferase gene in the pGEM-luci vector (Promega) and a BamHI-XhoI restriction fragment of pcDNA3 vector (Invitrogen) containing cytomegalovirus (CMV) promoter, human growth hormone first intron and SV40 early polyA signal sequence.

This vector can be constructed by an appropriate combination of the methods typically used in this field.

(3) Cell and cell culture

Human embryonic kidney cell (HEK-293), human cervical carcinoma cell (Hela-S3), murine melanoma cell (B16-F1) and murine fibroblast (NIH3T3) were purchased from American Type Culture Collection (ATCC). Each cell was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (50 units/ml) and streptomycin (50 μg/ml). Cells were grown and incubated in 5% CO_2-95% air atmosphere at 37°C.

(4) Transformant

Transformed cell, B16/L cell, was obtained by transfecting pCMV-luci vector into B16-F1 cells by Lipofect Amine Plus (Gibco BRL), screening the cells for clones resistant to antibiotics G418 (Geneticin, Gibco BRL) and selecting the objective cell from the clones based on the expression of luciferase activity as an index.

(5) Lipid

Cholesterol (Chol), egg yolk derived phosphatidylcholine (ePC) and egg yolk-derived sphingomyelin (eSph) were purchased from Sigma. Bovine brain-derived phosphatidylserine (bPS) was purchased from Avanti Polar Lipids. Dioleyl-phosphatidylethanolamine (DOPE) and dimethylaminoethanecarbamoyl-cholesterol (DC-Chol) were purchased from NOF.

(6) Preparation of HVJ

HVJ (Z strain, separated in Research Institute for Microbial Disease, Osaka University, Osaka, Japan) was grown in chorioallantoic fluid of an egg (10-day embryo) at 35.5°C. HVJ was collected by centrifugation at 27,000 g for 30 min and suspended in BSS. The HVJ suspension was preserved at 4°C before use. The RNA genome of HVJ was inactivated by UV irradiation (198 mJ/cm^2) immediately before use.

(7) Preparation of liposome

Each lipid was dissolved in chloroform at a concentration of 30 mM. For the preparation of cationic liposome (DC liposome), ePC, DOPE, eSph, Chol and DC-Chol were used at a molar ratio of 16.7:16.7:16.7:40:10. Each lipid solution (500 μl) was transferred into a glass tube and dried under reduced pressure to give a thin lipid film. This dry mixture of lipid was stirred for 30 sec in a Vortex mixer, re-dissolved in BSS or BSS/FR901228

solution (200 μl) containing vector DNA (200 μg), and stood for 30 sec. This step was repeated 8 times. To this admixture was added 800 μl of BSS and the mixture was passed through a cellulose acetate membrane (pore size, 0.45 μm), and the liposome on the membrane was collected with 500 μl of BSS. This mixture was passed through a cellulose acetate membrane (pore size, 0.2 μm) and collected in 2 ml of BSS.

(8) Preparation of HVJ liposome

To the DC liposome suspension prepared in the above (7) was added an HVJ suspension (30,000 hemagglutinating units), and the mixture was incubated for 10 min on ice. Then, this suspension was incubated at 37°C for 2 hr while stirring gently. The suspension was applied to a discontinuous sucrose density gradient (30% sucrose/BSS solution 6.5 ml), and centrifuged at 4°C, 62,800 g for 2 hr in a swing bucket rotor. The HVJ/liposome conjugate was present between BSS and 30% sucrose/BSS solution. The HVJ/liposome conjugate (hereinafter to be also referred to as HVJ-DC liposome) was collected, suspended in 2 ml of BSS, and subjected to the test using cell.

The trapping efficiency of the vector DNA into the final HVJ-DC liposome was approximately 60%.

(9) Measurement of luciferase activity of culture cell containing transgene

B16-F1 Cells (1×10^5 cells) were plated in each well (diameter 35 mm) of a 6 well tissue culture plate one day before gene transfer. The medium was changed to a medium containing FR901228 at a test concentration, and an HVJ-DC liposome (20 μl) including a pCMV-luci vector was added for gene transfer. After the lapse of the test time, the cells were collected and the state of expression of the luciferase gene was examined. For the test using B16/L cell, the same test as in B16-F1 cell was conducted except the absence of an HVJ-DC liposome. The expression of luciferase was examined using a luciferase assay kit (Promega). The total protein amount was measured by BCA method. The luciferase activity is expressed as a relative light unit (RLU)/total protein amount, or the ratio of RLU/total protein amount with the administration of FR901228 to RLU/total protein amount without the administration of FR901228. The test consisted

of three repeats of the same test in duplicate or triplicate.

(10) Determination of luciferase activity upon gene transfer into animal

Six-week-old male C57BL/6 mice were anesthetized by intraperitoneal administration of pentobarbital (1 mg), and B16-F1 cell or B16/L cell (1×10^6 cells) was transplanted subcutaneously in the back. Two weeks later, the transplanted tumor had grown to have a diameter of about 10 mm. In the case of B16/L cell, a BSS solution (200 μl) containing 0 μg, 27 μg or 54 μg of FR901228 was directly administered to the tumor. In the case of B16-F1 cell, an HVJ-DC liposome solution (200 μl) containing 0 μg or 27 μg of FR901228 and pCMV-luci vector was directly administered to the tumor. In both cases, the entire tumor lump was taken out 24 hr after the administration and quickly frozen in liquid nitrogen. After thawing, the tumor lump was minced, and completely pulverized homogeneously with a passive lysis buffer (500 μl) manufactured by Promega. After centrifugation, the supernatant was determined for luciferase activity according to the method described in the above (9).

2. Results

(1) Potentiation of transgene expression (*in vitro*) by FR901228 in gene transfer

Using an HVJ-DC liposome, a pCMV-luci vector was transduced into B16-F1 cells in the presence of FR901228 and potentiation of the luciferase gene expression was examined. FR901228 showed dose-dependent potentiation of the luciferase gene expression after 24 hr from gene transfection (Fig. 1A). The luciferase gene showed 26-fold and 52-fold potentiation of the expression in the presence of 10 nM and 100 nM FR901228, respectively. A similar test was performed using NIH3T3 cell. The expression of transduced luciferase gene in the NIH3T3 cell was low but by the presence of FR901228 at concentrations of 5 nM, 10 nM and 100 nM, the expression was potentiated 700-fold, 3400-fold and 5200-fold, respectively (Fig. 1B). Similar potentiation of the expression of transduced luciferase gene was observed in human embryonic kidney cell (HEK293) and cervical carcinoma cell (Hela).

In B16-F1 cell, significant potentiation of the expression of luciferase gene was achieved by the treatment in 100 nM

FR901228 for 30 min (Fig. 2).

From the above, it was clarified that remarkable potentiation of the expression of the transgene was derived by allowing action of FR901228 during the gene transfer.

(2) Reactivation (*in vitro*) by FR901228 of gene incorporated into genome

The B16/L cell incorporating luciferase gene under regulation of CMV promoter into the genome expresses the luciferase gene stably, while it is under transcription control of a host chromatin. Using this cell, reactivation of the transgene by FR901228 was examined. After 24 hr from the treatment with FR901228, dose-dependent potentiation of the expression of luciferase gene was found in B16/L cell (Fig. 3). The presence of FR901228 at concentrations of 5 nM, 10 nM and 100 nM resulted in the 7.7-fold, 15.3-fold and 24.7-fold potentiated expression, respectively. A significant potentiation of the expression of luciferase gene was observed from 6 hr after the treatment with 100 nM FR901228 and lasted for 24 hours thereafter.

From the above, it was clarified that FR901228 could derive sustained high expression after gene transfer by reactivation of the gene incorporated into the genome.

(3) Potentiation of expression (*in vivo*) of transgene by FR901228 in gene transfer

To a lump of B16-F1 tumor cell transplanted subcutaneously into C57BL/6 mice was administered an HVJ-DC liposome enclosing 27 μg FR901228 and pCMV-luci vector to examine potentiation of the transgene expression by FR901228. As a control, the luciferase gene alone was transduced into the tumor lump by HVJ-DC liposome but only a low level expression of the luciferase gene was observed. In contrast, when HVJ-DC liposome enclosing the luciferase gene with FR901228 was transduced into the tumor lump, the expression of the luciferase gene was potentiated 5.3-fold as compared to the absence of FR901228 (Fig. 4).

From the above, it was shown that FR901228 could derive remarkable potentiation of the transgene expression at the individual level by the action of FR901228 during gene transfer.

(4) Reactivation (*in vivo*) by FR901228 of the gene incorporated into the genome

To the lump of B16/L tumor cell transplanted subcutaneously into C57BL/6 mice was administered FR901228 to examine reactivation of the transgene by FR901228. When FR901228 (dissolved in BSS) was administered to the tumor lump in the amounts of 27 μg and 54 μg, the expression of the luciferase gene was potentiated 2.5-fold in both cases as compared to the absence of FR901228 (Fig. 5).

From the above, it was shown that FR901228 could derive remarkable potentiation of the sustained high transgene expression at an individual level by the reactivation of the gene incorporated into the genome.

Example 2

1. Test method

Eight-week-old male C57BL/6 mice were anesthetized by intraperitoneal administration of pentobarbital (1 mg), and B16-F1 cell (5×10^6 cells, see Example 1) was transplanted subcutaneously in the right flank. Seven days later, the transplanted tumor grew to have a diameter of about 10 mm. HVJ-DC Liposomes enclosing various administration amounts of FR901228 (0, 0.001, 0.01, 0.1, 1, 2.7, 5.4, 10 μg) and pCMV-luci vector were prepared according to Example 1. An HVJ-DC liposome solution (100 μl) containing 40 μg of pCMV-luci vector diluted with BSS was directly administered to the tumor. The entire tumor lump was taken out 24 hr after the administration and quickly frozen in liquid nitrogen. After thawing, the tumor lump was minced and homogenized with a passive lysis buffer (5-fold amount) manufactured by Promega. After centrifugation (12,000 rpm, 5 min), the supernatant was recovered and determined for luciferase activity according to the method described in Example 1(9). The measures are expressed in the mean of 5 mice.

2. Results

The results are shown in Fig. 6. Potentiation of the expression of luciferase gene was confirmed in the presence of FR901228.

Example 3

1. Test material

(1) Drugs

FR901228 isolated and purified according to the method

described in US Patent No. 4,977,138 was dissolved in balanced salt solution (BSS, 137 mM NaCl, 5.4 mM KCl, 10 mM Tris-HCl, pH 7.6) and used for the test (BSS/FR901228 solution).

Gancyclovir was purchased from TANABE SEIYAKU CO., LTD., dissolved in BSS and used.

(2) Vector

The pCMV-tk vector was constructed by inserting a 1.7 kb segment of thymidine kinase (HSV-TK) gene of I-type herpes simplex virus into the EcoRI/XhoI restriction site of pcDNA3 (Invitrogen).

2. Test method

Eight-week-old male C57BL/6 mice were anesthetized by intraperitoneal administration of pentobarbital (1 mg), and B16-F1 cell (5×10^6 cells/100 μl) was transplanted subcutaneously in the right flank. According to the schedule shown in the following Table 1, HVJ-DC liposomes [100 μl, enclosing both pCMV-tk vector (40 μg) and FR901228 (2.7 μg), one of them, or neither of them; each prepared according to Example 1] were directly injected into the tumor at 7 days after transplantation of the tumor cell. After 13 days after transplantation, namely, 6 days after injection of HVJ-DC liposome, BSS containing or not containing gancyclovir was intraperitoneally administered. The gancyclovir administration was performed once a day for 5 consecutive days in the dose of 1.5 mg/mouse/day.

Table 1

Group	HVJ-DC liposome		GCV	n
	Vector DNA	FR901228 (2.7 μg/body)		
A	pCMV-tk	+	+	9
B	pCMV-tk	-	+	7
C	BSS	-	+	7
D	pCMV-tk	-	-	6
E	pCMV-tk	+	-	7
F	BSS	+	-	7

Note: BSS under "Vector DNA" means addition of BSS as a control to liposome instead of pCMV-tk.

The volume of tumor was calculated from the following calculation formula twice a week after injection of the tumor cell.

$$\frac{\text{Long diameter} \times \text{short diameter}^2}{2}$$

3. Results

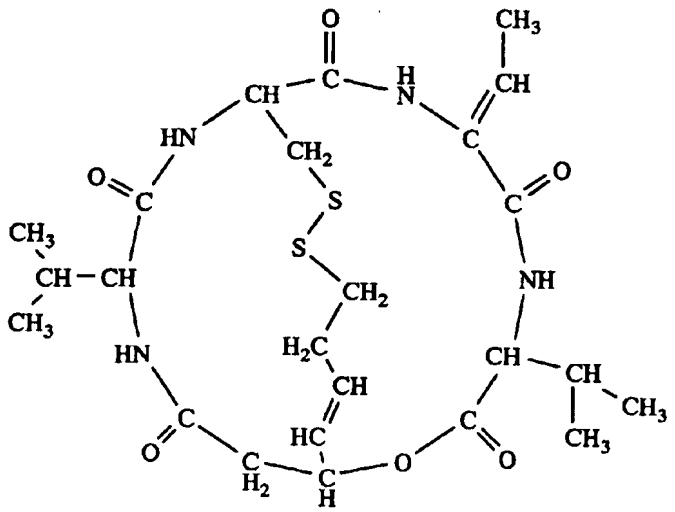
The results are shown in Fig. 7. The transfection of thymidine kinase gene and tumor growth inhibitory activity by the subsequent gancyclovir administration resulted in a marked tumor inhibitory activity in the presence of FR901228, as compared to the absence of FR901228. That is, the usefulness of the present invention was confirmed in a cancer gene therapy using a suicide gene and thymidine kinase gene/gancyclovir.

The expression potentiator and reactivation promoter of a transgene of the present invention, which contains FR901228 showing a histone deacetylase inhibitory activity or a salt thereof, exhibits not only *in vitro but in vivo* superior potentiation of the expression and promotion of reactivation of a transgene. Therefore, it can be advantageously used in clinical situations, particularly for a gene therapy.

This application is based on application No. 52582/2000 filed in Japan, the contents of which are incorporated hereinto by reference.

WHAT IS CLAIMED IS

1. An expression potentiator or reactivation promoter of a transgene, which comprises a compound of the formula (I)



(I)

or a salt thereof as an active ingredient.

2. The potentiator or promoter of claim 1, which potentiates expression or promotes reactivation of a transgene *in vivo*.

3. The potentiator or promoter of claim 1 or claim 2, which is a drug.

4. The potentiator or promoter of claim 3, wherein the drug is for a gene therapy.

FIG. 1 A

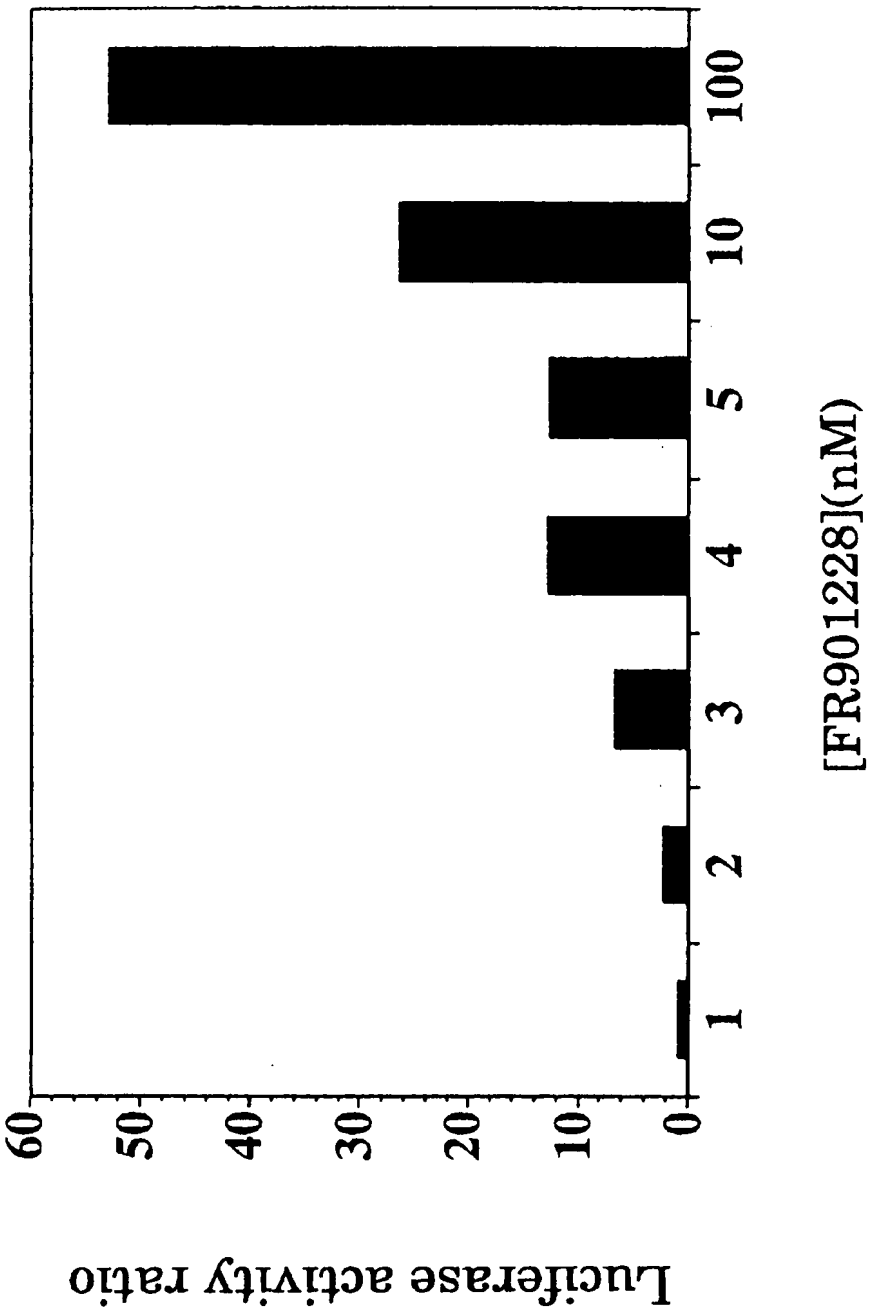


FIG. 1 B

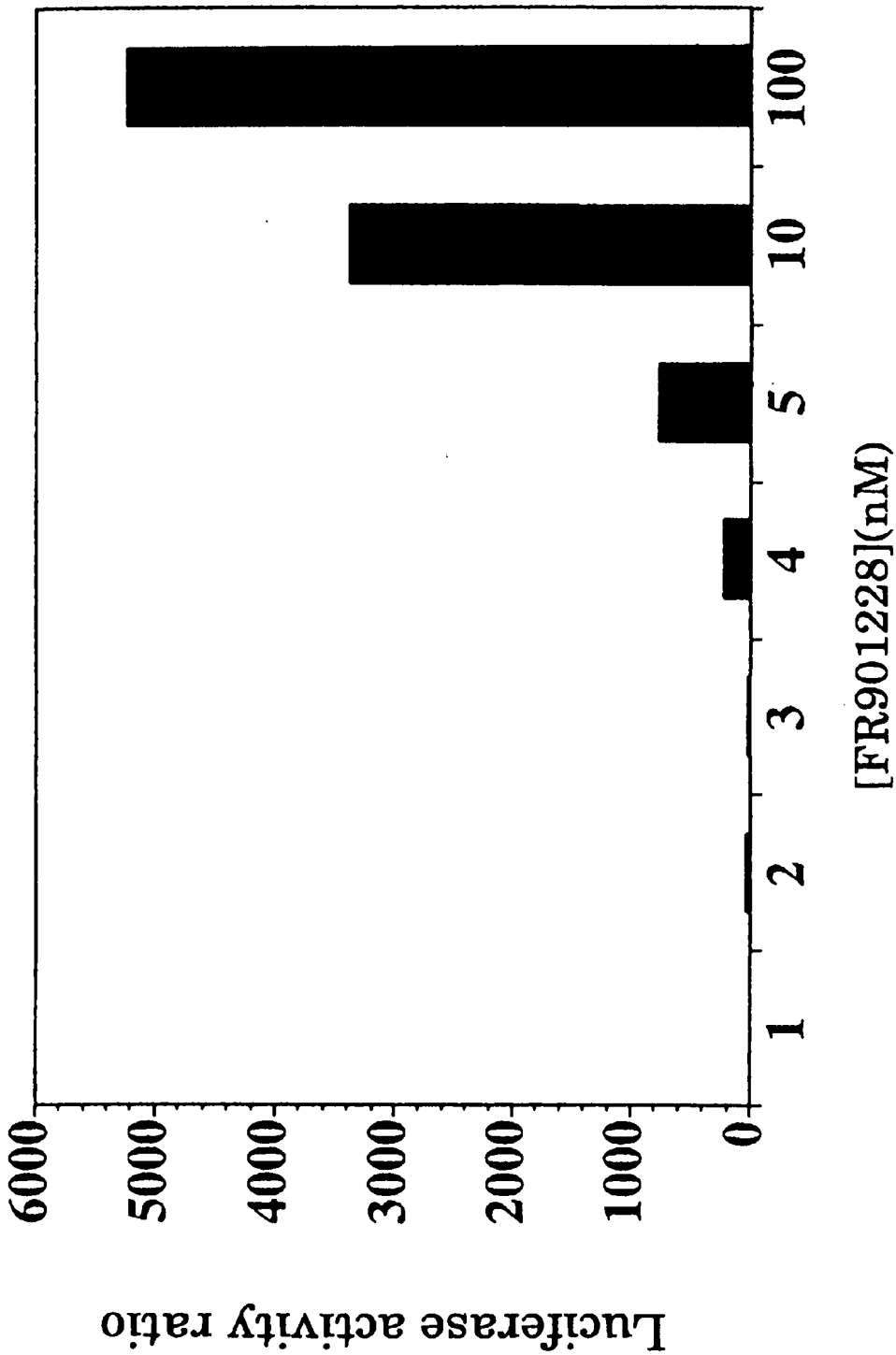

Luciferase activity ratio
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
10
100
[FR901228](nM)

FIG. 2

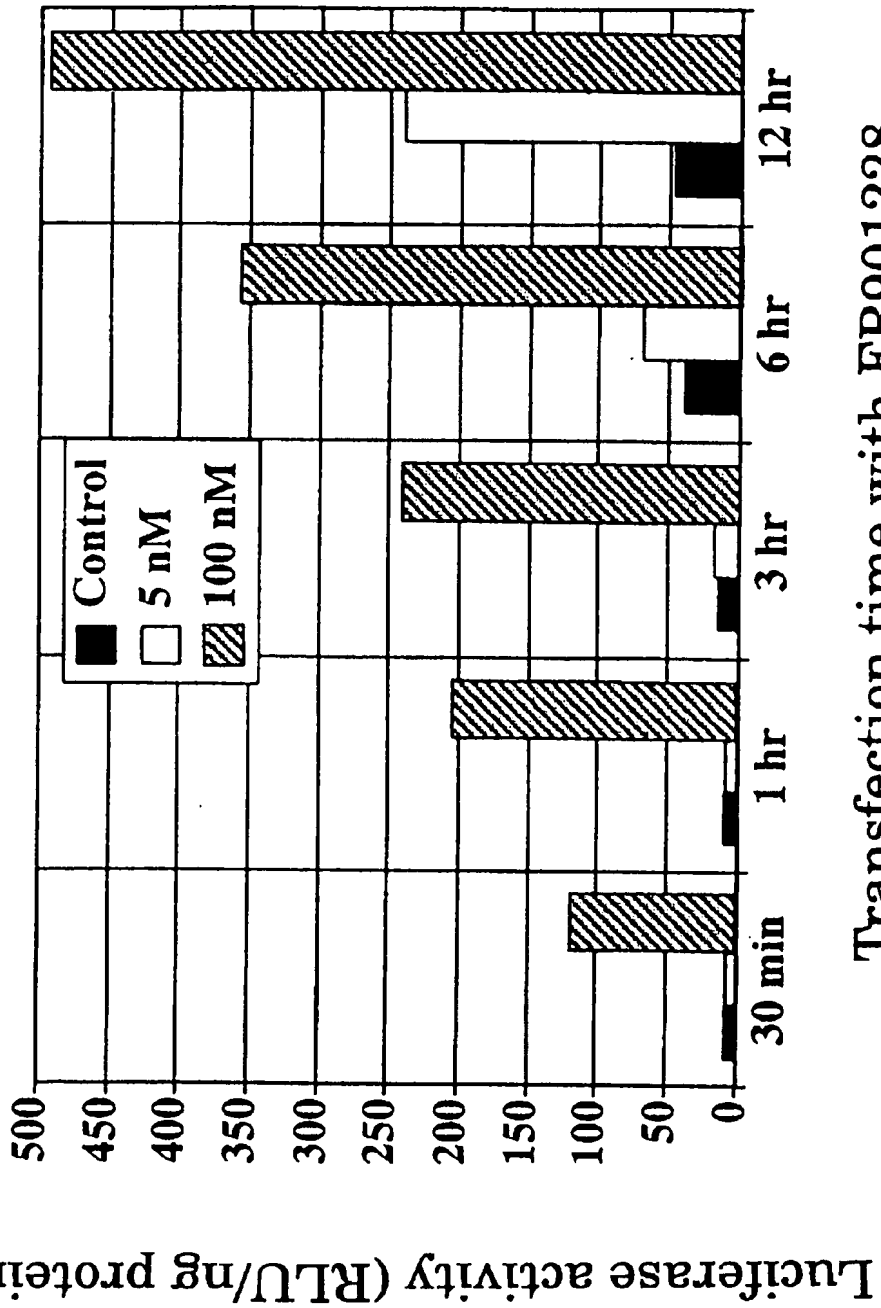

Luciferase activity (RLU/ng protein)
500
450
400
350
300
250
200
150
100
50
0
Control
5 nM
100 nM
30 min
1 hr
3 hr
6 hr
12 hr
Transfection time with FR901228

FIG. 3

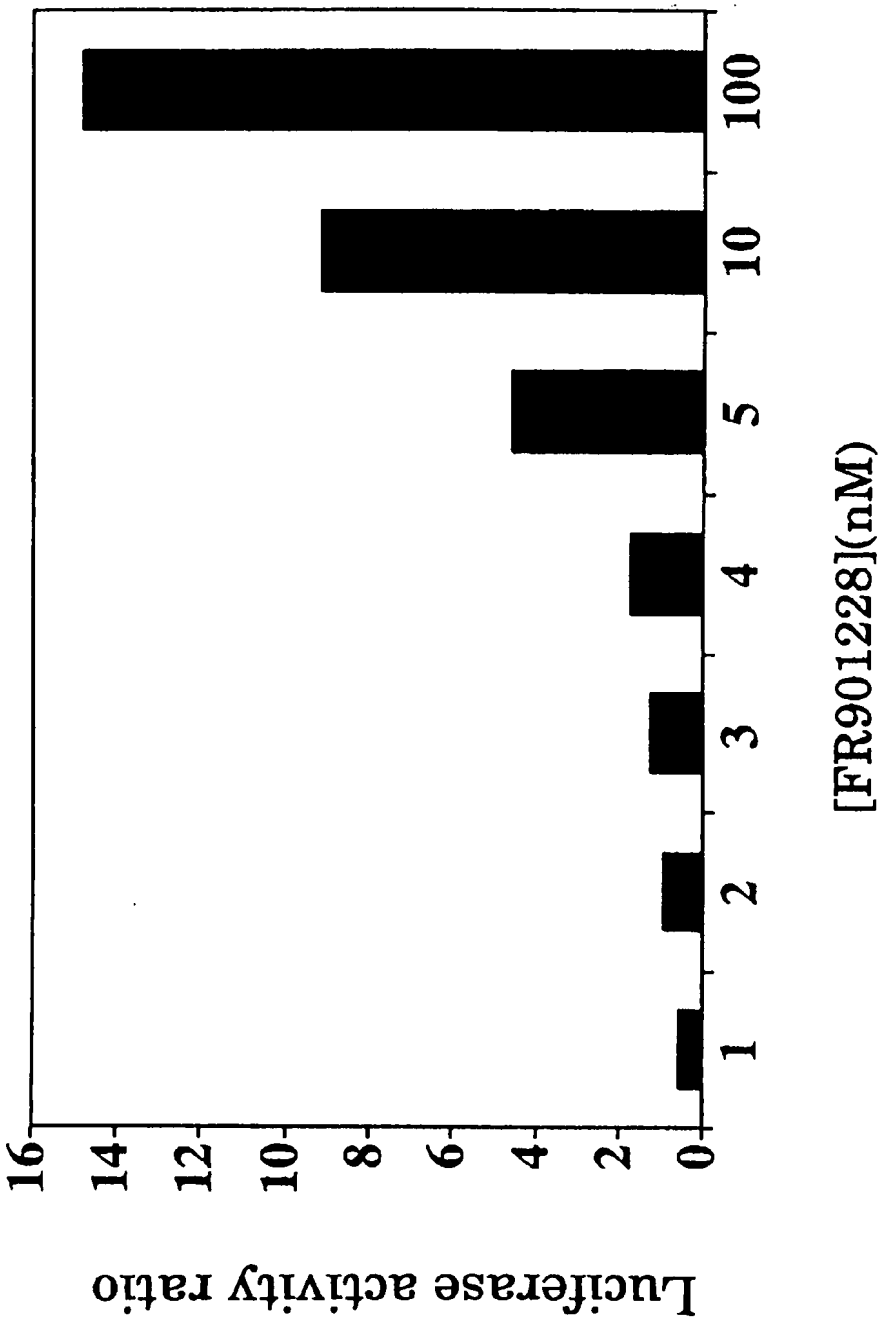

Luciferase activity ratio
0
2
4
6
8
10
12
14
16
1
2
3
4
5
10
100
[FR901228](nM)

FIG. 4

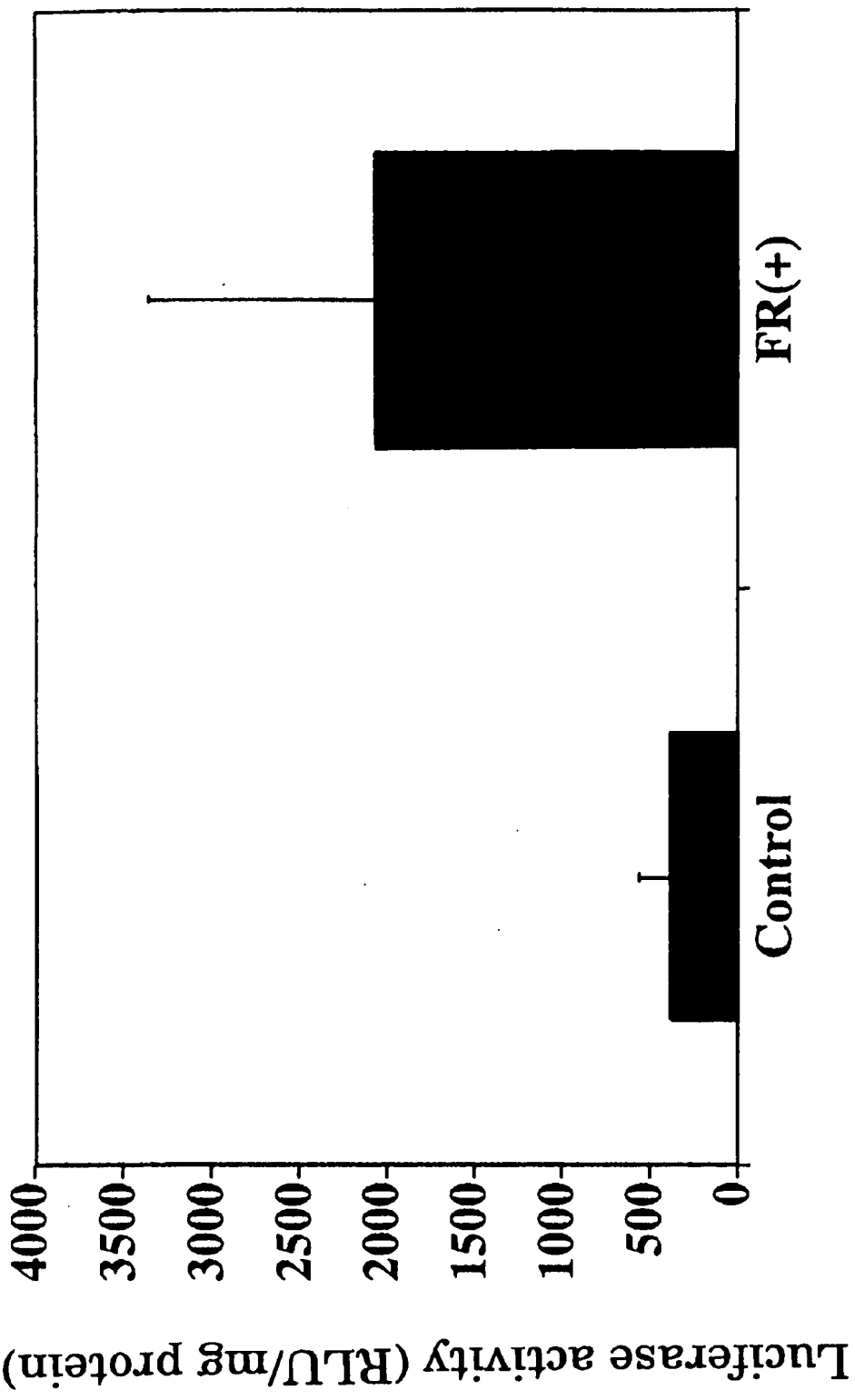

Luciferase activity (RLU/mg protein)
4000
3500
3000
2500
2000
1500
1000
500
0
Control
FR(+)

FIG. 5

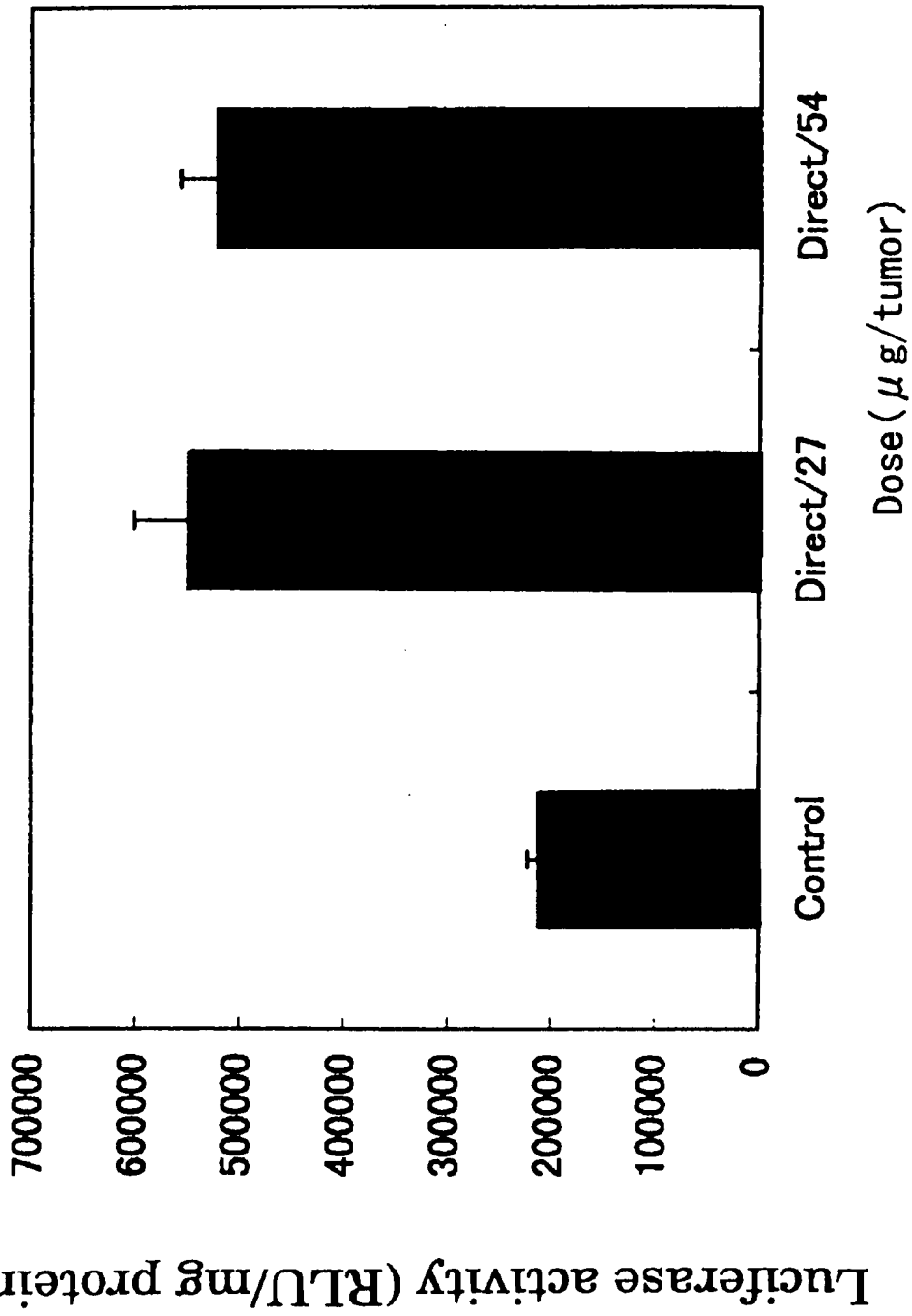

Luciferase activity (RLU/mg protein)
700000
600000
500000
400000
300000
200000
100000
0
Control
Direct/27
Direct/54
Dose (μg/tumor)

FIG. 6

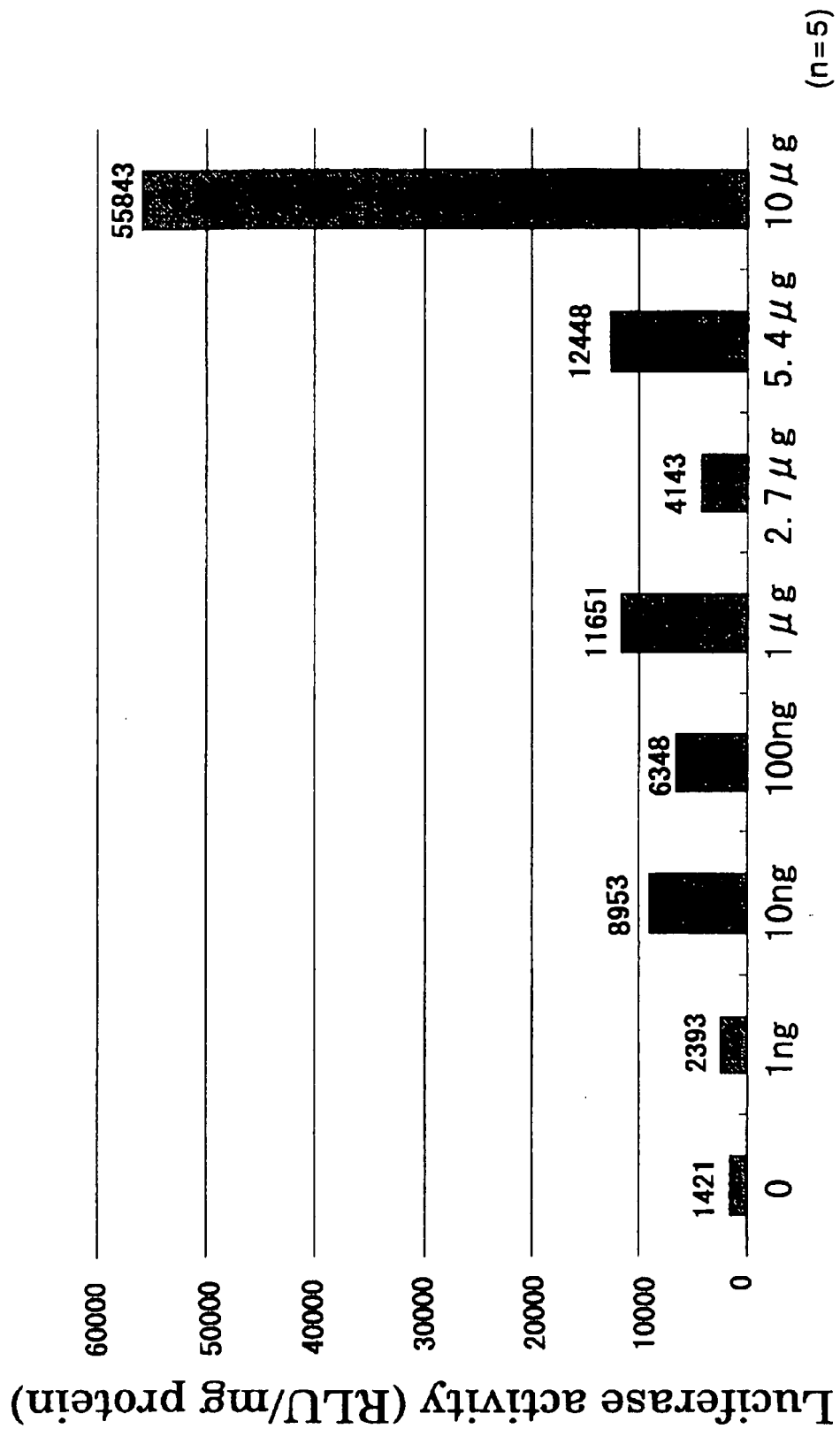
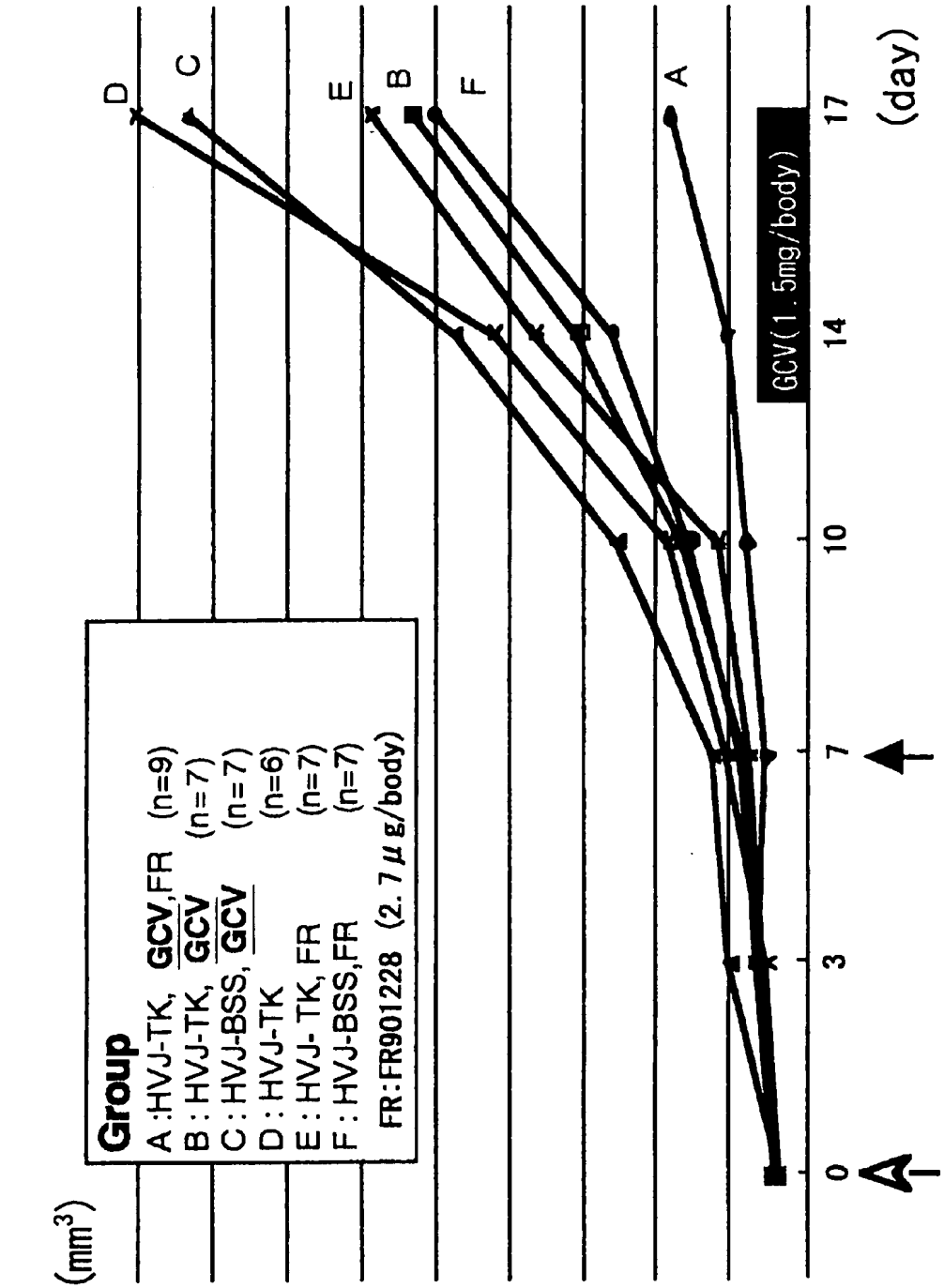

Luciferase activity (RLU/mg protein)
60000
50000
40000
30000
20000
10000
0
1421
2393
8953
6348
11651
4143
12448
55843
0
1ng
10ng
100ng
1μg
2.7μg
5.4μg
10μg
(n=5)
FR901228 dose/body

FIG. 7


Tumor volume (mm3)
4500
4000
3500
3000
2500
2000
1500
1000
500
Group
A :HVJ-TK, GCV,FR (n=9)
B : HVJ-TK, GCV (n=7)
C : HVJ-BSS, GCV (n=7)
D : HVJ-TK (n=6)
E : HVJ- TK, FR (n=7)
F : HVJ-BSS,FR (n=7)
FR:FR901228 (2.7μg/body)
D
C
E
B
F
A
GCV(1.5mg/body)
0
3
7
10
14
17
(day)
A
Cell injection
HVJ-DNA